Strength of attachment affects survival of *Salmonella* on inoculated cantaloupe treated with sanitizers

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Difficulty in removing or inactivating bacterial human pathogens on whole cantaloupe surfaces is due both to the surface irregularities (crevices and pits in the netting) and to increasing time intervals between contamination and sanitizer treatments, presumably resulting in stronger attachment and/or biofilm formation.

The objective of this study was to determine the relationship between strength of attachment of *Salmonella* serovars to melon surfaces and the effectiveness of sanitizer treatments during a 24 h storage period.

Whole melons were inoculated at ~4.5 $\log_{10}$ CFU/cm^2 and stored at 25°C for up to 168 h before washing with water, chlorine (200 ppm) or hydrogen peroxide (2.5%). The ability of *Salmonella* serovars to resist removal by washing with water and killing by chlorine and hydrogen peroxide was compared at 20 min, 30 min, and 2, 6, 9 and 24 h post inoculation. Washing with water within 30 min after inoculation led to a significant ($p<0.05$) removal of a cocktail of *Salmonella* Poona strains (1.5 $\log_{10}$ unit reduction), but was totally ineffective thereafter. The efficacy of the sanitizer treatments in eliminating *Salmonella* from the melon surface was also dependent on the time interval between inoculation and treatment. Between 2 and 24 h storage, the strength of attachment for *Salmonella* on melon surfaces varied slightly among serovars, but generally increased from approximately 0.2 to 0.9. A 3 $\log_{10}$ unit reduction was achieved when chlorine and hydrogen peroxide treatments were applied up to 2 h after inoculation. Above 6 h, population reductions were approximately only 2 $\log_{10}$ units. Populations of *Salmonella* transferred to fresh-cut pieces during rind removal survived and grew during storage at 5, 10, 15 and 20°C for up to 10 days. However, storage at 5°C suppressed growth of *Salmonella* on fresh-cut pieces for up to day 8 when prepared within 2 h after inoculation and sanitizer treatment. A higher number of *Salmonella* was recovered in fresh-cut pieces prepared at day 7 immediately after sanitizing treatment. The results of this study indicate that *Salmonella* rapidly becomes strongly attached to the cantaloupe rind surface and that strongly attached bacteria are more difficult to remove by washing with water or inactivation with sanitizer treatments.

Keywords: *Salmonella*, strength of attachment, cantaloupe, sanitizer

1 Introduction

The ability of pathogenic bacteria to adhere to surfaces of fruits and vegetables continues to be a potential food safety problem of great concern to the produce industry. Surface structure and physiological characteristics of bacteria and surface structure and physical properties of the substratum, in this case the melon rind, play a major role on how and where bacteria may attach. The surface of cantaloupe is comprised of a meshwork of tissue commonly referred to as the "net" [1]. The presence of the raised net tissue gives the surface an inherent roughness which may favor microbial attachment and hinder detachment. Cantaloupe contaminated with various *Salmonella* serovars has been the cause of several outbreaks of foodborne illness in the U.S. The last three recorded U.S. cantaloupe-related outbreaks of salmonellosis have been due to *Salmonella* Poona.
Bacterial attachment to surfaces is influenced not only by cell surface charge [2] and hydrophobicity [3;4;5;6], but also by the presence of particular surface appendages such as flagella and fimbriae (pili) as well as extracellular polysaccharides [7;8]. Flagella, fimbriae, outer membrane proteins, and extracellular polysaccharide may influence bacterial attachment to plant surfaces [9]. Ukuku et al., [10] reported that bacteria attached on cantaloupe surfaces for more than 2 days were difficult to remove by washing treatment. Ukuku and Sapers [11] reported transfer of *Salmonella* Stanley inoculated on cantaloupe surface to fresh-cut pieces during rind removal. A better understanding of bacterial adhesion to cantaloupe is needed for the development of more effective washing treatments to control microorganisms on melon surfaces and their transfer to fresh-cut pieces. In this study, we investigated the effect of attachment strength on survival and removal of *Salmonella* populations on whole cantaloupe surfaces washed with water or sanitized with chlorine or hydrogen peroxide.

2 Materials and methods

2.1Bacterial strains, growth conditions, and inoculum preparation.

Sixteen *Salmonella* strains were used in this study: *Salmonella* Stanley H0558, *Salmonella* Newport H1275, *Salmonella* Anatum F4317, *Salmonella* Infantis F4319, *Salmonella* Poona RM2350, *Salmonella* Hidalgo 02-517-2, *Salmonella* Typhimurium 45, *Salmonella* Gaminara 02-615, *Salmonella* Mbandaka 00-916, *Salmonella* Poona G-91-1595, *Salmonella* Poona 953, *Salmonella* Poona 348, *Salmonella* Poona 418, *Salmonella* Michigan, *Salmonella* Oranienburg 389, and *Salmonella* St. Paul 02-517. Bacteria were maintained on Brain Heart Infusion Agar (BHIA, BBL/Difco, Sparks, MD) slants held at 4°C. Prior to use, each culture was subjected to two successive transfers by loop inocula to 5 ml Brain Heart Infusion Broth (BHIB, BBL/Difco). A final transfer of 0.2 ml was made into 20 ml BHIB with incubation at 36°C for 18 h under static conditions. Bacterial cells were harvested by centrifugation (10,000 × *g*, 10 min) at 4°C, and the cell pellets were washed in salt-peptone [0.85% NaCl, 0.05 % Bacto-peptone (BBL/Difco)]. The cell pellets were used to prepare two different types of inoculum as stated below. The first inoculum type consisted of the individual bacterial strains at 10^8 CFU/ml. The second inoculum type consisted of a mixture containing strains of all five strains of *Salmonella* Poona at approximately 1.4×10^8 CFU/ml per strain. Both types of inoculum were prepared in 3 L of 0.1 % (w/v) peptone-water.

2.2 Inoculation of cantaloupe.

Unwaxed whole cantaloupes (Western shippers) purchased from a local produce distributor were allowed to come to room temperature (~20°C) overnight before being inoculated. Individual cantaloupes were submerged in 3liters of each of the inocula (~18°C) and agitated by stirring with a glove covered hand for 10 min and then treated as stated below.

2.3 Washing treatments.

A commercial bleach containing 5.25% sodium hypochlorite (NaOCl, Clorox®, Clorox Company, Oakland, CA, USA), was diluted in sterile water to obtain a wash solution containing 200 ppm of chlorine. The pH was adjusted to 6.4 ± 0.1 by adding citric acid (Sigma Chemical Co., St Louis, MO, USA). Free chlorine in the solution was determined with a chlorine test kit (Hach Co., Ames, IA, USA). A second wash solution was prepared from a 30% stock solution of hydrogen peroxide (Fisher Scientific, Suwanee, GA, USA) that was diluted to 2.5% in sterile water. Washing treatments were performed by totally submerging the melons in 3 l of sterile tap water, or water containing 200 ppm chlorine, or 2.5% hydrogen peroxide. Melons were manually rotated for 5 min to assure complete contact of surfaces with the wash solution. Washed melons were placed on crystallizing dishes inside a biosafety cabinet to dry for 1 h.

2.4 Attachment experiments.

Cantaloupes inoculated with individual or cocktails of strains were washed as described above after intervals of 20, 40, 60 min or 1 to 7 days post inoculation with storage at 5 to 25° C. Bacterial cells in the wash water (loosely attached) and those remaining on the melon surfaces were enumerated as described below. The population remaining on the melon surface after washing (water) treatment was described as strongly attached bacteria. The strength of attachment (S_R) values were calculated as (strongly attached bacteria)/(loosely + strongly attached bacteria) as previously reported [6; 12]. The S_R value represents the percentage of the total bacterial population strongly attached to the cantaloupe.

2.5 Microbiological examination.

Plugs (n = 40) of cantaloupe rind (2.2 cm) weighing approximately 25 g total were cut with a sterile stainless steel cork borer and blended (Waring commercial blendor with 75 ml of 0.1% peptone-water at speed level 5 for 1 min.) *Salmonella* was enumerated on XLT4 agar (BBL/Difco, Sparks, MD) with incubation at 35°C for 48 h. For comparison, a pure culture of *Salmonella* was plated on XLT4 agar (BBL/Difco), incubated as above, and run parallel with the samples. Selected black or black-centered colonies from the agar plates were confirmed to be *Salmonella* according to the FDA Bacteriological Analytical Manual following conventional biochemical methods [13] as well as serological assays using latex agglutination (Oxoid, Ogdensburg, New York).

3 Results and discussion

3.1 Strength of attachment and removal by washing with water.

The relationship between S_R-values and log reduction for a cocktail of *Salmonella* Poona strains inoculated on cantaloupe surface and then washed with water following storage at 25° C, is shown in Figure 1.

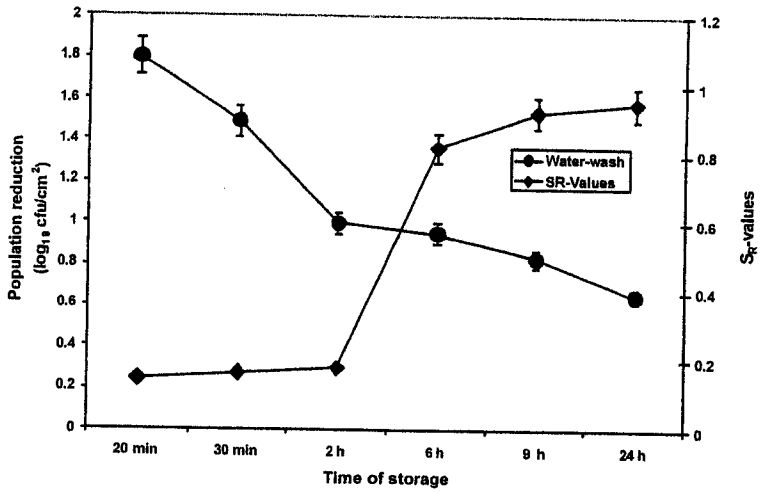


Fig. 1

Washing with water was most effective when applied within 20 min of inoculation resulting in a population reduction of 1.8 log_{10} units of *Salmonella* from the melon surface. Log reductions gradually decreased with increasing storage time before treatment to about 0.6 log_{10} at 24 h. After 2 h post inoculation, reduction of the *Salmonella* population by washing with water was not significant ($p>0.05$). The attachment strength (S_R-values) for *Salmonella* increased abruptly from 0.2 to 0.9 at 2 h to 6 h, followed by a slight increase between 6 and 24 h.

3.2 Strength of attachment and removal by sanitizer treatment.

The relationship between S_R-values and log reduction for a cocktail of *Salmonella* Poona strains inoculated on cantaloupe surface and then sanitized with chlorine or H_2O_2 following storage at 25°C is shown in Figure 2.

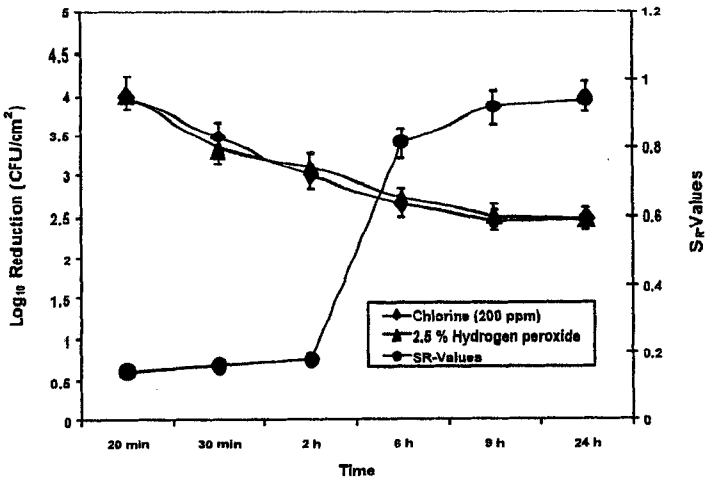


Fig. 2

Sanitizer treatments with chlorine or H_2O_2 were most effective in reducing populations of *Salmonella* when applied within 20 min after inoculation resulting in an ~ 4 log reduction for *Salmonella* on the cantaloupe surface. Population reductions decreased gradually with time of storage with a 2.5 log unit reduction at 24 h. Differences between sanitizers in the effect of post inoculation storage on population reductions obtained were not significantly ($p>0.05$) different. The extensive raised netting on the surface of cantaloupe melon no doubt provides numerous microbial attachments sites and helps to protect attached microbes from being washed from the surface, killed with sanitizers and possibly from environmental stresses such as UV radiation and desiccation. Surface irregularities such as roughness, crevices, and pits have been shown to increase bacterial adherence and reduce the ability of washing treatments to remove bacterial cells [14–16].

S_R-values of *Salmonella* populations on whole cantaloupe surfaces after storage of melons at 25°C for up to 2 h and log reductions due to chlorine treatments are shown in **Table 1**.

Salmonella Serovar/strain	S_R-value (30 min)	S_R-value (1 h)	S_R-value (2 h)
Poona G-91-1595	0.152 ± 0.012[a] (4.18±0.12)[b]	0.158 ± 0.021 (3.46±0.15)	0.167 ± 0.012 (2.96±0.20)
Stanley H0558	0.177 ± 0.023 (3.95±0.15)	0.185 ± 0.013 (3.48±0.16)	0.197 ± 0.016 (2.85±0.14)
Poona RM2350	0.177± 0.021 (4.23±0.16)	0.185 ± 0.020 (3.68±0.15)	0.198 ± 0.022 (3.04±0.18)
Newport H1275	0.184± 0.032 (3.60±0.12)	0.189± 0.022 (3.09±0.22)	0.199± 0.017 (2.69±0.20)
Hidalgo 02-517-2	0.162 ± 0.012 (4.22±0.18)	0.176 ± 0.013 (3.89±0.13)	0.183 ± 0.015 (3.08±0.15)
Typhimurium 045	0.179 ± 0.021 (3.89±0.21)	0.181 ± 0.016 (3.14±0.15)	0.189 ± 0.023 (2.79±0.13)
Gaminara 02-615	0.168± 0.021 (3.96±0.21)	0.178± 0.017 (2.88±0.14)	0.188± 0.017 (2.58±0.14)

Poona 953	0.177 ± 0.022 (3.67±0.14)	0.185 ± 0.022 (2.97±0.15)	0.193 ± 0.021 (2.72±0.21)
Poona 348	0.174 ± 0.020 (3.85±0.17)	0.188 ± 0.022 (3.19±0.16)	0.197 ± 0.016 (2.79±0.12)
Poona 418	0.173 ± 0.012 (3.89±0.15)	0.185 ± 0.016 (3.12±0.16)	0.198 ± 0.018 (2.79±0.15)
Michigan	0.178 ± 0.015 (3.49±0.22)	0.187 ± 0.021 (2.82±0.16)	0.208 ± 0.015 (2.58±0.14)
St. Paul 02-517	0.185 ± 0.022 (3.55±0.21)	0.197 ± 0.021 (3.08±0.21)	0.199 ± 0.014 (2.85±0.16)
Oranienburg 389	0.178 ± 0.018 (3.89±0.12)	0.185 ± 0.011 (2.87±0.13)	0.198 ± 0.011 (2.48±0.14)
Mbandaka 00-916	0.170 ± 0.013 (3.68±0.14)	0.183± 0.015 (2.89±0.12)	0.189 ± 0.015 (2.63±0.16)
Anatum F4317	0.172 ± 0.018 (3.85±0.15)	0.186 ± 0.038 (3.16±0.14)	0.194 ± 0.015 (2.86±0.15)
Infantis F4319	0.174 ± 0.020 (3.87±0.17)	0.185± 0.022 (2.99±0.14)	0.187 ± 0.020 (2.57±0.12)

[a]Values not in parentheses are means ± SD of three experiments with duplicate determinations per experiment. The average populations of individual strains of *Salmonella* recovered from the cantaloupe surface after inoculation was ~ 4.5 $\log_{10}$ CFU/cm^2.
[b]Values in parentheses represent log reductions after treatment with 200 ppm Cl_2 for 5 min.

The strength of attachment for all strains averaged 0.17 at 30 min and increased to an average of 0.19 after storage at 25°C for 2 h. *Salmonella* serovars Michigan and St. Paul had the highest S_R-values indicating stronger attachment on melon surfaces, and were slightly more resistant to the sanitizer treatment than the rest of the strains tested. Log reductions after treatment with Cl_2 and time of storage were negatively correlated. The shorter the storage time, the greater the log reduction.

S_R-values of *Salmonella* populations on whole cantaloupe surfaces after storage of melons at 25°C for 24, 72 and 168 h and log reductions due to chlorine treatments are shown in **Table 2**

Salmonella Serovar/strain	S_R-value (24 h)	S_R-value (72 h)	S_R-value (168 h)
Poona G-91-1595	0.822 ± 0.032[a] (2.88±0.12)[b]	0.856 ± 0.022 (2.46±0.12)	0.861 ± 0.012 (2.41±0.22)
Stanley H0558	0.815 ± 0.028 (2.85±0.18)	0.861 ± 0.023 (2.31±0.23)	0.885 ± 0.018 (2.35±0.18)
Poona RM2350	0.803 ± 0.021 (3.03±0.12)	0.862 ± 0.022 (2.72±0.21)	0.884 ± 0.020 (2.54±0.20)
Newport H1275	0.874± 0.052 (2.60±0.12)	0.881± 0.022 (2.51±0.22)	0.889± 0.012 (2.29±0.22)
Hidalgo 02-517-2	0.796 ± 0.018 (3.16±0.18)	0.806 ± 0.016 (2.76±0.16)	0.803 ± 0.015 (2.68±0.25)
Typhimurium 045	0.819 ± 0.022 (2.59±0.22)	0.834 ± 0.012 (2.34±0.12)	0.859 ± 0.026 (2.29±0.16)
Gaminara 02-615	0.798± 0.024 (2.76±0.24)	0.808± 0.027 (2.58±0.17)	0.818± 0.014 (2.48±0.14)
Poona 953	0.817 ± 0.027 (2.87±0.17)	0.847 ± 0.022 (2.57±0.16)	0.862 ± 0.020 (2.62±0.20)

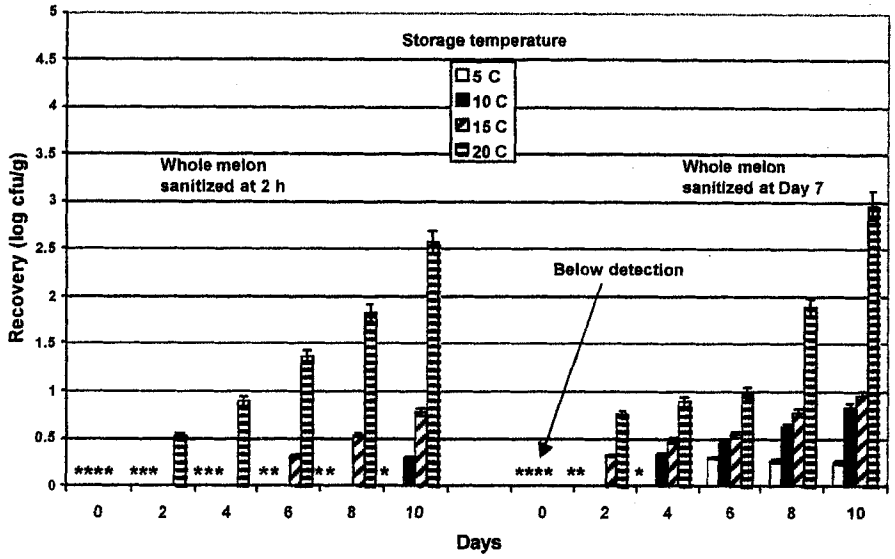
Poona 348	0.871 ± 0.025 (2.85±0.15)	0.908 ± 0.022 (2.69±0.12)	0.901 ± 0.015 (2.49±0.15)
Poona 418	0.882 ± 0.032 (2.83±0.12)	0.922 ± 0.021 (2.62±0.21)	0.928 ± 0.018 (2.28±0.18)
Michigan	0.911 ± 0.020 (2.59±0.20)	0.942 ± 0.023 (2.22±0.13)	0.948 ± 0.015 (2.28±0.18)
St. Paul 02-517	0.905 ± 0.026 (2.75±0.26)	0.938 ± 0.023 (2.48±0.23)	0.955 ± 0.022 (2.35±0.12)
Oranienburg 389	0.783 ± 0.018 (2.83±0.18)	0.795 ± 0.013 (2.65±0.13)	0.798 ± 0.014 (2.68±0.14)
Mbandaka 00-916	0.800 ± 0.016 (2.70±0.16)	0.835 ± 0.012 (2.55±0.15)	0.840 ± 0.019 (2.40±0.19)
Anatum F4317	0.852 ± 0.028 (2.82±0.18)	0.866 ± 0.038 (2.66±0.18)	0.874 ± 0.018 (2.74±0.18)
Infantis F4319	0.842 ± 0.021 (2.84±0.21)	0.854 ± 0.026 (2.54±0.16)	0.872 ± 0.022 (2.72±0.12)

[a]Values not in parentheses are means ± SD of three experiments with duplicate determinations per experiment. The average populations of individual strains of *Salmonella* recovered from cantaloupe surface after inoculation was ~ 4.5 log CFU/cm^2.

[b]Values in parentheses represent log reductions after treatment with 200 ppm Cl_2 for 5 min.

As the strength of attachment is increased due to storage at 25°C, the efficacy of the sanitizer treatment is reduced. The population of *Salmonella* on cantaloupe surfaces was reduced by 2 $\log_{10}$ unit at 168 h. Again, serovars Michigan and St. Paul that had higher S_R-values were slightly more resistant to the sanitizer treatment than the rest of the strains tested.

The population of a cocktail of *Salmonella* Poona strains on fresh-cut cantaloupe pieces prepared from inoculated melons sanitized with 200 ppm chlorine either 2 h or 168 h after inoculation and storage for various times and temperatures in shown in Figure 3.



No *Salmonella* were recovered on Day 0 of storage following fresh-cut processing prepared within 2 h and 168 h of inoculating the whole melon and sanitizer treatment. The population of *Salmonella* on fresh-cut pieces was greater with increasing storage temperature and time. Growth was detected earlier on fresh-cut pieces prepared from inoculated melons held 7 days prior to sanitizing and processing, compared to melons held only <2 h, suggesting greater transfer during fresh-cut preparation with the longer post-inoculation storage time before treatment with the sanitizer.

In conclusion, the strength of attachment of a cocktail of *Salmonella* Poona strains increased with time of storage at 25°C and was negatively correlated with population reductions after washing with water or sanitizer treatments (Figures 1 and 2).

The attachment strength for all individual *Salmonella* strains on the cantaloupe rind was low at 20 min to 2 h post-inoculation storage at 25°C, but increased greatly after storage for 24 to 168 h (Tables 1 and 2). As for the cocktail of *Salmonella* Poona strains, higher S_R-values due to longer storage times for the individual strains of *Salmonella* resulted in lowered efficacy of water washes or sanitizer treatments (Tables 1 and 2). Treatment of inoculated whole melons with 200 ppm Cl_2 was not effective in elimination of *Salmonella* Poona from the the surface and the pathogen was transferred to fresh-cut pieces. Continuous storage at 5°C is important to delay growth of the surviving pathogenic bacterial cells.

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